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NITROSOGUANIDINE MUTAGENESIS AND CHROMOSOMAL REPLICATION IN STAPHYLOCOCCUS AUREUS

Robert A. Altenbern

MAY 1969

DEPARTMENT OF THE ARMY
Fort Detrick
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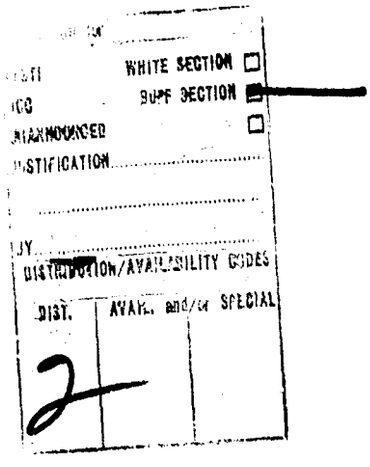

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Robert A. Altenbern

Medical Bacteriology Division
BIOLOGICAL SCIENCES LABORATORIES

Project 1B061102B71A

May 1969

NITROSOGUANIDINE MUTAGENESIS AND CHROMOSOMAL REPLICATION IN STAPHYLOCOCCUS AUREUS

ABSTRACT

The number of mutations induced by nitrosoguanidine per 10^6 survivors is the same for exponential cells as for cells with resting, complete chromosomes. During synchronous chromosomal replication, no peak of mutations per million survivors appears at the time of duplication of the genes examined. It was concluded that for Staphylococcus aureus the replicating region of the chromosome is no more susceptible to nitrosoguanidine mutation than is the resting chromosome.

I. INTRODUCTION*

Recent investigations in several laboratories have led to varied methods for determining gene locations on the chromosome of several organisms. Wolf, Newman, and Glaser[1] developed an elegant method for mapping the Escherichia coli K-12 chromosome; a considerably different method was devised by Helmstetter[2] for E. coli B/r. Cerda-Olmeda, Hanawalt, and Guerola[3] were able to derive a map of E. coli by nitrosoguanidine mutation during synchronous chromosomal replication; our own efforts[4,5] in mapping the Staphylococcus aureus chromosome involved similar methodology.

It was shown that the replication point in the E. coli chromosome was significantly more amenable to nitrosoguanidine mutagenesis than the non-replicating regions.[3] The report of this phenomenon led me to search for parallel behavior in S. aureus. To date, the evidence indicates that the replicating point of the S. aureus chromosome is no more mutable by nitrosoguanidine than is the non-replicating portion of the chromosome. In this context, S. aureus appears to be qualitatively different from E. coli.

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II. MATERIALS AND METHODS

Single auxotrophs of two strains (Smith and Maybush) of S. aureus were isolated following exposure of the parent type to nitrosoguanidine and were employed throughout this study. The synchronous chromosomal replication method for mapping the S. aureus genome was carried out as previously described[5] except that Albimi Brucella broth* replaced trypticase soy broth. Total viable counts were determined on plates of trypticase soy agar. Mutagen-induced prototrophs were detected and counted on minimal medium whose composition has already been described.[4]

Resting cells with completed chromosomes were prepared as follows: 10 ml of overnight culture in Albimi Brucella broth were added to 100 ml of fresh medium of the same composition. The culture was shaken at 37 C for 3 hours, at which time phenyl ethyl alcohol was added (0.40%) and incubation was continued at 30 C for 2 hours without shaking in a water bath. At the end of this incubation, the cells had ceased DNA synthesis and presumably possessed completed chromosomes. A 5-ml sample of these cells was centrifuged, the cells were resuspended in 5 ml of saline containing 200 μg nitrosoguanidine per ml and incubated at 30 C for 20 minutes. After this mutagen exposure, the cells were diluted in saline and plated on minimal agar and on trypticase soy agar to determine the number of induced reversions to prototrophy and the total survivors, respectively. Exponential cells were grown for 5 hours on a shaker at 37 C following inoculation of 5 ml of Albimi Brucella broth with 0.05 ml of an overnight culture in the same medium. These cells were then centrifuged and subjected to the same mutagen exposure as the resting cells.

III. RESULTS

The results of determination of the mutations per 10^6 survivors for three loci in each of two strains for exponential cells and for cells with resting chromosomes are presented in Table 1. It is clear that in strain Maybush the number of mutants per 10^6 survivors for exponential cells is the same as that for resting cells at each of the three loci. In strain Smith the mutations per 10^6 survivors in resting cells seem to be consistently higher than for exponential cells. These data contrast markedly with those reported for E. coli,[3] in which the mutants per 10^6 survivors for exponential cells were 4 to 5 times greater than the same parameter determined for cells with resting chromosomes.

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* Albimi Laboratories, Flushing, New York.

TABLE 1. FREQUENCY OF MUTATIONS PER 10^6 SURVIVORS INDUCED IN EXPONENTIAL CELLS AND CELLS WITH RESTING CHROMOSOMES FOR TWO STRAINS OF S. AUREUS

	Mutations per 10^6 Survivors[a/]		
	pan	rib	trp
	Strain Maybush		
Exponential	13,100	6.8	10.9
Resting	14,680	5.3	10.1
	Strain Smith		
Exponential	36	0.75	156
Resting	55	1.9	210

a. pan = pantothenate; rib = riboflavin; trp = tryptophan.

Determination of total mutants, total survivors and, thus, mutants per 10^6 survivors for each of three loci for strain Smith during synchronous chromosomal replication led to the results presented in Figure 1. There is no peak of mutants per 10^6 survivors at the time of duplication of the gene in question. Total survivors seem to increase slightly with time, although other earlier data[5] showed that there is no increase in number of cells before exposure to nitrosoguanidine.

The point in time of pronounced increase in mutants per 10^6 survivors at various loci in E. coli during synchronous chromosomal replication,[3] designated the gene duplication time, has not been observed in S. aureus. The reasons for such a sharp contrast between these two organisms can be at present only a matter of conjecture. Cerda-Olmeda et al.[3] speculated that, in E. coli, opening of the double-stranded chromosome during replication increased the mutability of that open region. In S. aureus this region does not exhibit increased mutability and may be partially protected by the replicase itself or by other aspects of the mechanics of replication.

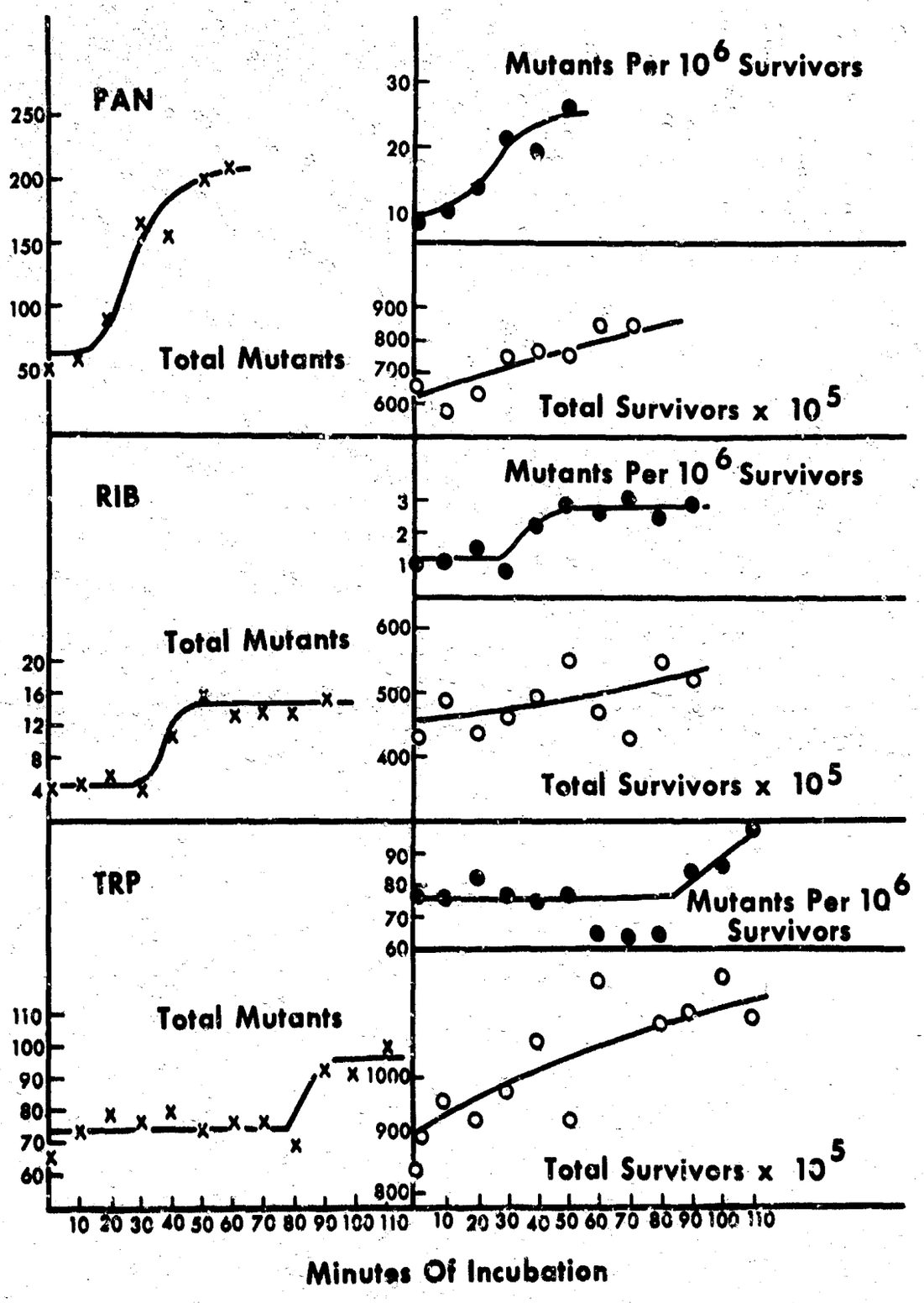

FIGURE 1. Total Mutants and Total Survivors During Synchronous Chromosomal Replication Mapping of Auxotrophs of S. aureus, strain Smith.

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14. Key Words

*Staphylococcus aureus
*Nitrosoguanidine
*Chromosomes
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Auxotrophs

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